

Coccidian intestinal parasites: Diagnosis and treatment

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Abstract: Intestinal coccidian parasites such as *Cryptosporidium*, *Isospora*, *Cyclospora* and *Sarcocystis* have gained importance as emerging pathogens in the era of AIDS. Several protozoan infections that had previously been considered rare are now much more commonly recognized in the immunocompromised group and, more importantly many new species of parasitic protozoa have been recognized as human parasites. All coccidian parasites have a complex life-cycle involving asexual, sexual and sporogenous cycles, and details are discussed here.

Cryptosporidium

The coccidian parasite *Cryptosporidium* causes a disease called cryptosporidiosis. It infects the epithelium of gastrointestinal tract to produce self-limiting in immunocompetent but potentially life-threatening diarrhoea in immunocompromised patients, especially in those with AIDS. It is responsible for 2.2% (range 0.26%-22%) of cases of diarrhoea in immunocompetent persons in developed countries and 6.1% (range, 1.4%-41%) in developing countries. It has been reported in up to 7% of children with diarrhoea in developed countries and up to 12% in developing countries.¹ Cryptosporidial infection in immunocompromised patients, especially those with AIDS, leads to persistent watery diarrhoea, malabsorption and weight loss, and tends to involve the biliary tract, pancreas, stomach and the middle ear. In developed countries, it occurs in 14% (range 6%-70%) of patients with AIDS and diarrhoea; in developing countries, it occurs in 24% (range 8.7%-48%) of such patients. The proportion of general population excreting oocysts is 1%-3% in developed countries and 10% in developing countries.¹ In India, HIV serosurveillance 1996-1997 showed an HIV positivity rate of 21.07 per 1000 population and *C. parvum* infection in 27.5% of these patients.² A study conducted at the Post-Graduate Institute of Medical Education and Research, Chandigarh has documented this infection in 10.8% of HIV/AIDS patients.³ In Manipur, 94.4% of HIV-positive drug abusers were infected with *C. parvum*.⁴

There are 10 species of this parasite but *C. parvum* is the only species responsible for human infections. Further, it has been categorized into two genotypes the human type 1 and bovine type 2. Most human infections occur directly by the faecal-oral route or indirectly by fomites. Zoonotic transmission from cattle and sheep to humans is known and the latter are considered as important reservoirs. *Cryptosporidium* oocysts may be found in all types of water including untreated surface water, filtered swimming pool water and even chlorine-treated or filtered drinking water. This is a growing concern since waterborne outbreaks have been reported worldwide.⁵ The 1993 outbreak in

Milwaukee, U.S.A. resulted in the death of several immunocompromised patients and caused illness in many previously healthy persons.⁶

Diagnosis

The simplest tool available is microscopic examination of the stool, sputum or bile. These specimens should be submitted as fresh material or in 10% formalin or sodium acetate-acetic acid-formalin (SAF) preservative. Fixed specimens are recommended because the cysts are highly infectious and resistant to disinfectants. Potassium dichromate solution is routinely used as a storage medium to preserve oocyst viability.⁷

Stool examination

A minimum of three samples collected on alternate days need to be examined. This is especially true for formed stool specimens, which contain fewer cysts than diarrhoeal stools. The stool samples are examined after concentration by either Sheather sugar flotation technique, zinc sulphate (specific gravity 1.18), or saturated salt solution (specific gravity 1.27), or sedimentation techniques such as formol-ether or formalin-ethyl acetate. To detect *Cryptosporidium* cysts, it is advisable to centrifuge the stool specimen at more than 500 g for at least 10 minutes.⁷

Unstained wet mount preparation: Faeces may be examined using phase-contrast or bright-field microscopy. However, the oocysts are considered infectious and resistant to routinely used disinfectants. Hence, stained smears are better than wet preparations.

Staining techniques: Most recommended stains for *Cryptosporidium* oocysts cannot be performed on stools commonly preserved in polyvinyl alcohol fixative. The widely used techniques include modified acid-fast staining and negative staining.^{8,9} Less common staining methods include auramine-rhodamine and acridine orange.¹⁰ All stained preparations should be examined using a high dry (40x) or oil immersion objective.

Immunofluorescent (IF) antibody: Procedures employing

Cryptosporidium-specific polyclonal or monoclonal antibodies have been developed and may provide the most sensitive method for the diagnosis of cryptosporidiosis.¹¹ The sensitivity of acid-fast (AF) immunofluorescence for detecting *C. parvum* oocysts in human stool has been reported to be 10 000 oocysts per g of watery stool while in formed stools 50 000 or 500 000 oocysts are required for a positive IF or AF test, respectively.¹²

Autofluorescence: The green autofluorescence of the oocysts under violet or ultraviolet (UV) light illumination can be used to detect *Cryptosporidium* in cell cultures. However, due to similarly fluorescing spores and yeast cells of identical size,¹³ identification of oocysts in faecal samples using only autofluorescence is not recommended.

Serodiagnosis

Specific IgG or IgM antibodies have been detected by enzyme-linked immunosorbent assay (ELISA) using crude oocyst preparations as antigens. However, detection of specific antibodies only indicates prior exposure. Antigen detection: Immunoassays using uncentrifuged, fresh, frozen or fixed faecal material can be used for antigen detection. Currently, enzyme immune assay kits are available. The sensitivity and specificity of such tests are in the range of 90%-100%. Some of these, particularly the combination of direct fluorescence product used to identify both *Giardia lamblia* and *Cryptosporidium* cysts, are being widely used in water testing and outbreak situations.⁷

Flow cytometry

Flow cytometric assays for the detection of *Cryptosporidium* oocysts in stool have been shown to be ten times more sensitive than conventional IF assays.¹⁴

Molecular techniques

Application of molecular techniques has contributed greatly to the understanding of the genetic diversity among *Cryptosporidium* isolates. Polymerase chain reaction (PCR), with its various modifications, has been used by many groups of workers. A sensitivity of 97% in nested PCR assays to detect *Cryptosporidium* DNA in fixed, paraffin-embedded tissues and water samples has been reported.¹⁵ An important advantage of PCR is its ability to directly differentiate between genotypes of *Cryptosporidium*.

Treatment

The clinical course of cryptosporidiosis depends largely on the immune status of the host and, therefore, the treatment options vary. Generally, asymptomatic and immunocompetent persons need no specific therapy. Supportive therapy with oral or intravenous fluids and electrolyte replacement helps to correct the dehydration that accompanies acute diarrhoea and the patient recovers spontaneously. In children, spiramycin (100

mg/kg/day) may shorten the duration of oocyst excretion and diarrhoea, although the data on this aspect are conflicting. In patients with AIDS, the best treatment is improvement of immune function with highly active antiretroviral therapy (HAART), which also helps to resolve *Cryptosporidium* infection. If HAART is not possible, combination therapy with an antimicrobial and an antidiarrhoeal agent helps. More than 100 antimicrobial agents have been tested so far, but none has been found to be consistently curative. Some clinical improvement and decrease in oocyst shedding has been seen with the non-absorbable aminoglycoside paramomycin (1 g twice daily) and azithromycin (600 mg daily) for 4 weeks followed by paramomycin monotherapy for an additional 8 weeks.¹⁶ Nitazoxanide, a nitrothiazole benzamide compound with a wide spectrum of activity against protozoa, helminths and bacterial pathogens, appears to have some efficacy against human *Cryptosporidium*. Severely immunocompromised AIDS patients with refractory cryptosporidiosis may show variable response to letrozuril. Ongoing trials with immunological intervention hold promise. The best treatment for biliary cryptosporidiosis in patients with AIDS is still HAART. It can help to resolve infection but may not eradicate the organism from the biliary tract. For patients with pain or cholangitis associated with papillary stenosis, endoscopic sphincterotomy may provide striking symptomatic relief.⁵

Control and prevention

Most conventional water treatment methods do not effectively remove or kill all oocysts. Thus, preventive measures include health education about proper handwashing and boiling or filtration of water (filter pores < 1µm diameter).

Cyclospora

Both immunocompromised as well as immunocompetent persons can be infected. The mode of transmission of infection is water or food. Waterborne outbreaks of diarrhoeal disease have been reported from different regions throughout the world. A number of food items, especially fresh fruits, vegetables and dishes containing items (raspberries, baby lettuce and basil) have been implicated. Two outbreaks had been reported in the USA and Canada in 1996 and 1997 and were related to the import and consumption of Guatemalan raspberries.¹⁷ Prevalence rates of *C. cayetanensis* in HIV patients in India have been reported to range from 3% to 5.2%.^{3,18,19} The infection is seasonal, and occurs particularly during the warm and rainy seasons. The signs and symptoms of the disease include explosive diarrhoea, abdominal cramps, vomiting, anorexia, fatigue and weight loss. In immunocompetent patients, the diarrhoea is prolonged but self-limiting and lasts for 1-6 weeks. In immunocompromised patients, the diarrhoea is even more protracted. *Cyclospora* resides in the upper small bowel and can cause villus atrophy, crypt hyperplasia and inflammatory changes.²⁰

Diagnosis

Diagnosis of cyclosporiasis is mainly dependent on the detection of oocysts or its antigens/DNA in faecal samples or, less commonly, in the jejunal aspirates of infected persons.

Unstained wet mount preparation

C. cayetanensis can be detected by examining an unstained normal saline preparation under a microscope at a magnification of 440X. The oocysts appear as non-refractile, round, hyaline structures containing an arrangement of refractile membrane-bound globules. Measurement of the oocyst is essential to differentiate it from other coccidia, especially *Cryptosporidium*. The oocysts of *Cyclospora* measure 8-10 µm in diameter, while *Cryptosporidium* oocysts measure 4-6 µm in diameter and *Isospora* oocysts are 22-33x10-19µm in size.²⁰

Staining methods

With acid-fast stains, oocysts show variable staining (compared to *Cryptosporidium* oocysts where the majority of oocysts take up the stain), with the colour varying from deep red to pink to unstained; some may contain granules or have a bubbly appearance. It has been observed that in fresh faecal samples, most of the oocysts show good staining, while in older samples, the majority may be decolorized. A strong decolorizer should not be used. Sulphuric acid solution (1%) is recommended for the staining of all coccidian parasites. A more consistent and rapid staining method based on a modified safranin technique stains oocysts of *Cyclospora* a brilliant reddish-orange colour. Other stains that have been used are auramine-O, methylene blue, trichrome, iron-haematoxylin and methanamine silver. These staining procedures are usually more cumbersome and less sensitive.

Auto fluorescence

Primary fluorescence of oocysts is a feature that appears to be unique to *Cyclospora* and *Isospora* oocysts. This method allows the detection of oocysts even if they are covered with faecal debris. The colour of the oocysts appears as neon blue at the excitation wavelength of 330-380 nm and green at 450-490 nm.

The oocysts of *Cyclospora*, excreted in the faeces, sporulate outside the host in about 2 weeks resulting in two sporocysts, each containing four sporozoites. The oocysts can also be induced to sporulate in the presence of 5% potassium dichromate.¹⁴

Molecular methods

There are only a few studies which have tried to use PCR for the diagnosis of cyclosporiasis. Though this may not find application in the field at present, it may help in the epidemiological investigation of oocysts in foods such as raspberries, etc.

Treatment

The drug of choice is trimethoprim/sulphamethoxazole (TMP-SMX) at a dose of 160/800 mg twice daily for 7 days. This eradicates the organisms and decreases symptoms. In patients with AIDS, the treatment recommended is similar. However, maintenance therapy with TMP-SMX 3 times a week or sulphadoxine 500 mg + pyrimethamine 25 mg once a week is given to prevent relapse. Ciprofloxacin 500 mg twice a day for 10 days has been reported to be a reasonable alternative in patients unable to tolerate TMP-SMX.²¹

Control and prevention

It is extremely difficult to control and prevent cyclosporiasis because of the limited ability to detect low infective doses of oocysts that may contaminate products such as raspberries. The oocysts are resistant to conventional water treatment procedures such as chlorination and therefore, boiling of drinking water is recommended. Fresh fruits and vegetables should be thoroughly washed and/or peeled before consumption. The role of handwashing cannot be overemphasized.

Isospora

All species of *Isospora* are obligate intracellular parasites, mainly in vertebrates. In humans, two species of *Isospora* have currently been identified, *I. belli* and *I. natalensis*. The latter was reported from South Africa in the early 1950s and apparently there are no further reports in human beings. *I. belli* has a worldwide distribution, with most cases occurring in the tropics. Enteric infections with *I. belli*, once considered rare, are increasingly being recognized in patients with AIDS. It produces chronic, intermittent secretory-like diarrhoea that leads to dehydration. In patients with AIDS, recurrence of symptoms is a common manifestation despite treatment. Prevalence rates in India have been reported to range from 2.5% to 31%.^{3,18,19} Animal sources of human infection have not been identified and infection is usually thought to occur by ingestion of oocyst-contaminated food or water. Histologically, *I. belli* infection can induce villus atrophy and crypt hyperplasia.

Diagnosis

Specific identification of the organism requires examination of the faeces, which may contain the infective oocysts. However, examination of fresh material, either as a direct smear or as concentrated material by wet preparation, is recommended rather than permanent stained smears. The oocysts are either pale and transparent and can be easily missed. A biopsy may be positive while no organisms may be seen in the stool because of the small numbers. Oocysts of *I. belli* are elongate, ellipsoidal and are 20-33 x 10-19 µm in size. The oocysts are either unsporulated or partially sporulated and can sporulate in less than 24 hours.²⁰

Unlike the sporulated oocysts of *Cyclospora*, both sporocysts and oocysts of *Isospora* autofluoresce a neon-blue colour when illuminated by UV light of 330-380 nm wavelength and viewed under an epifluorescence microscope. The organisms may also be demonstrated by acid-fast staining.

Treatment

The treatment of choice is TMP-SMX (160-800 mg) 4 times a day for 10 days followed by twice a day for 3 weeks. In patients with AIDS, maintenance therapy with the same drug is given. In patients allergic to sulphonamides, pyrimethamine alone (50-75 mg daily) has cured infections. Ciprofloxacin has been found to be a reasonable alternative for patients unable to tolerate TMP/SMX.²¹ Nitazoxanide has also been found to be effective in eliminating *I. belli*.

Sarcocystis spp.

Humans serve as a definitive host for *Sarcocystis hominis* and *Sarcocystis suihominis* and also as accidental intermediate hosts for several unidentified species of *Sarcocystis*. *Sarcocystis* spp. cause a disease called sarcocystosis, the symptoms of which vary with the species causing the infection. It may present as intestinal or muscular sarcocystosis. Infection is acquired by ingesting uncooked beef (*S. hominis*) containing sarcocysts. *S. hominis* is only mildly pathogenic compared to the more pathogenic *S. suihominis* (found in pork). The oocysts and sporocysts of *Sarcocystis* are discharged over a period of several months; they are resistant to freezing and drying, and spread by invertebrate transport hosts. There is little or no immunity to reshedding of sporocysts, which are passed in the infective form.⁵

Diagnosis

Diagnosis of intestinal sarcocystosis is easily made by faecal examination. Oocysts are colourless, thin-walled and contain two elongated sporocyst, each of which contains four elongated sporozoites and a granular sporocyst residuum. The thin oocyst wall often ruptures, releasing the sporocysts in the intestinal lumen from where they are passed in the faeces. The sporocysts that are recovered in stool are oval, measuring 9-16 µm and contain four mature sporozoites and the residual body. Sporocysts or oocysts of *Sarcocystis* are shed fully sporulated in the faeces whereas those of *I. belli* are often shed unsporulated. Till date, it has not been possible to distinguish one species of *Sarcocystis* from another.

Treatment

No specific therapy is known to be effective for *Sarcocystis* infections.

Control and Prevention

Prevention includes adequate cooking of pork and beef.

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